

# Combining Confocal SR Micro-XRF and Absorption Microtomography for studying a Full Factorial Zn Exposure Experiment on *Daphnia magna*

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The study of the effects of the presence of transition metals such as Co, Cu, Ni and Zn on the health of pelagic and benthic invertebrates is an important research topic in the field of environmental toxicology. The freshwater crustacean *Daphnia magna* is a frequently used model organism to investigate the mechanisms of toxicity of the metals mentioned above. Historically, it has often been difficult to link bioaccumulation to toxic effects in daphnids because experimental techniques using total organism digestion cannot distinguish the accumulation in critical tissues from whole body accumulation. In previous work, we offered an elegant solution to this problem in illustrating a quantitative comparison between two differently exposed *Daphnia* samples using 2D dynamic scanning  $\mu$ -XRF, coupled with laboratory absorption microtomography [Ref. 1].

A full factorial ecotoxicological experiment was designed in which the importance of different Zn exposure routes on *Daphnia magna* was investigated; the results of the experiment are illustrated in Fig. 1 (left). In the *daphnia* series feeding on clear algae, a clear optimum in aquatic Zn concentration for reproduction can be observed; an additional amount of dietary Zn however, results in a decrease of reproduction. In order to relate these effects to metal accumulation, dorsoventral Zn element distributions of a series of samples were required through the region of gill tissue, gut and eggs in a non-destructive manner. Dynamic scanning confocal  $\mu$ -XRF was preferred to obtain virtual slices since  $\mu$ -XRF computed tomography ( $\mu$ -XRF CT) would require longer measuring times and is more elaborate in terms of sample alignment, data reconstruction and quantification. For more information about the experimental set-up, we refer to another contribution [Ref. 2].

The results of the analysis are shown in Fig 1 (right); a clear increase of Zn can be observed with higher aquatic Zn concentrations in both series. The effect of dietary Zn however remains less clear, a more thorough data analysis on sub areas using image segmentation (e.g. K-means clustering) is being performed to improve on data quality and interpretation. An estimation of the volume concentration was made using the elemental yields of a Bovine Liver Standard (NIST SRM 1577), assuming a confocal analyzing volume of  $10 \times 10 \times 10 \mu\text{m}^3$ . After performing a depth scan through the reference material in order to determine the boundaries and thickness, the reference material was measured just below the surface in order to have negligible self-absorption effects.

Figure 2 (left) illustrates another powerful capability of confocal  $\mu$ -XRF: to investigate smaller areas of metal accumulations within earlier obtained larger “overview” element distributions. First, a dorsoventral section was obtained in order to have a general overview of the elemental distributions. Subsequently, different 3D sub regions of interest such as eggs (1), gut and gill tissue (2) were analysed in more detail in using smaller scanning steps (e.g. 5 or 10  $\mu\text{m}$  instead of 20  $\mu\text{m}$ ) or longer measuring times per voxel (e.g. 3 s instead of 1 s). A powerful aspect of confocal  $\mu$ -XRF is that the elemental distributions in different planes of interest can easily be analysed. In (3) a sagittal section through the very same sample is visualised, in which the colours red, green and blue are again the scaled Ca, Zn and Fe intensities, proportional to the elemental concentrations. The same sample was also analysed using laboratory  $\mu$ -CT, producing 3D density/absorption coefficient data with which the dorsoventral and sagittal elemental distributions were coupled. This allows to combine the 2D elemental information with the 3D internal structure/morphology of the sample, as illustrated in Fig. 2 (right). Due to recent improvements on the scanning software at beamline L, it will become possible to obtain full 3D correlation between elemental and absorption datasets on the micrometer scale.

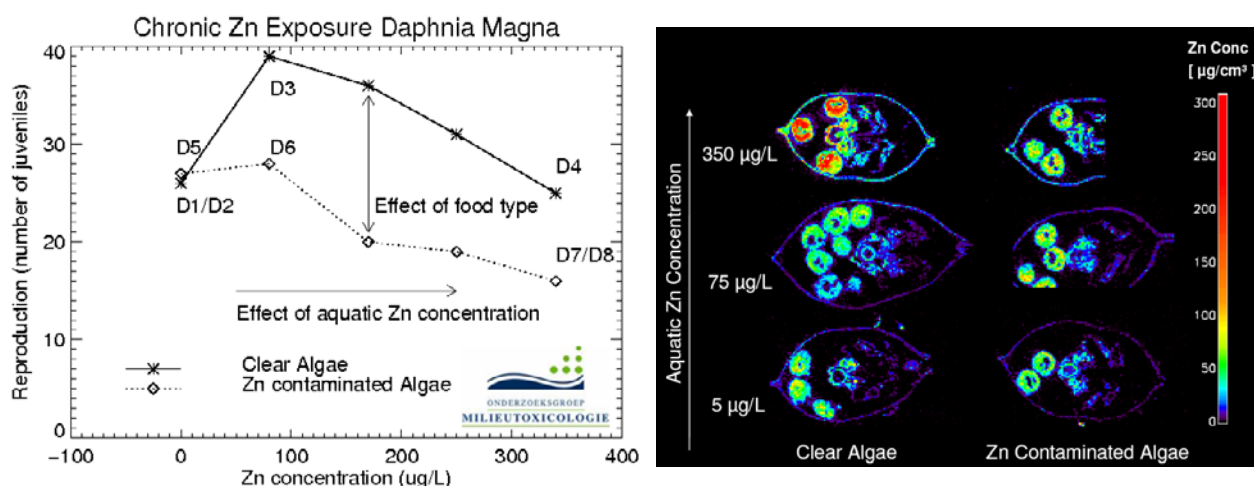


Fig. 1: The left image illustrates the effect of a chronic Zn exposure on *Daphnia magna*. The right image shows the dorsoventral Zn element distributions of sample D2 to D6 obtained by confocal  $\mu$ -XRF (20  $\mu$ m step size). A colorbar on the right indicates the Zn volume concentration in  $\mu$ g/cm<sup>3</sup>.

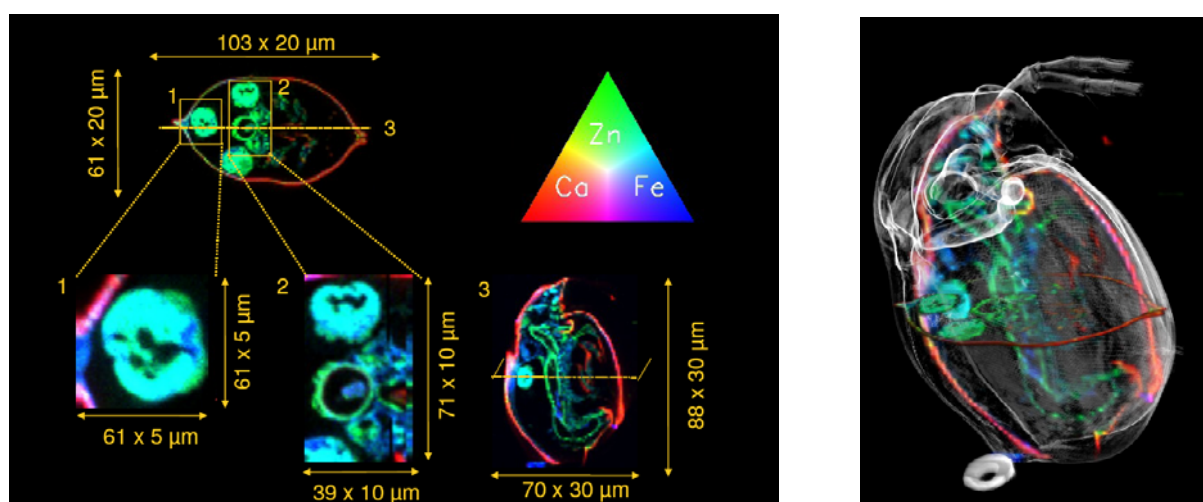


Fig. 2: The left image shows a detailed confocal  $\mu$ -XRF analysis of *Daphnia* D4 (see fig.1 for exposure conditions). The topleft image shows a dorsoventral overview scan (20 $\mu$ m stepsize). Subregions were analysed using smaller scanning step sizes (Image 1: egg, 5  $\mu$ m step size; Image 2: gut, 10  $\mu$ m step size). Image 3 shows a sagittal overview scan (30  $\mu$ m step size). The right image shows a 3D absorption CT set of *Daphnia* D4 combined with the dorsoventral and sagittal  $\mu$ -XRF scans.

## References:

- [1] SR-X-Ray Fluorescence (2D/CT) and Laboratory Absorption Microtomography reveal Tissue Specific Metal Distribution in *Daphnia Magna*, Hasylab Annual Report 2007, in press
- [2] Three-dimensional elemental imaging by means of synchrotron radiation micro-XRF: developments and applications in environmental chemistry. Anal. Bioanal. Chem. 2008, in press
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